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POST-MORTEM METABOLISM

AND MEAT QUALITY PROPERTIES

IN FIVE LAMB MUSCLES

A Dissertation

submitted in partial fulfilment

of the requirements for the Degree of

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by

Ziqian Feng

Lincoln University

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Abstract of a Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Science in Food Innovation.

POST-MORTEM METABOLISM AND MEAT QUALITY PROPERTIES IN FIVE LAMB MUSCLES

by

Ziqian Feng

The objective of this study was to analyze pH changes within five lamb muscles and correlate those to the physical and biochemical characteristics of the muscles, meanwhile, to understand what drives the changes and determine the strength of the correlation in pH changes with meat quality properties. Semimembranosus (SM), semitendinosus (ST), longissimus dorsi (LD), infraspinatus (IS), and supraspinatus (SS) from six animals were analyzed. Muscles from the left side of the carcasses were excised at 4 hours post-mortem to measure the fiber type by electrophoresis, while muscles from the right side of the carcasses were used to measure pH and temperature at 1.5, 6, 8, and 24 hours, and color was measured after the muscles were excised at 24 hours. After that, muscles from the right side were aged for 7 days and checked for cooking loss and tenderness. SM, ST, and LD muscles presented predominantly type-Ⅱa and -Ⅱx fibers, while SS was more abundant in type-Ⅰ fiber, and IS presented more type-Ⅰ fiber than SM, ST, and LD, but less than SS. Temperature of all muscles decreased and reached 3 ℃. All the muscles followed the normal trend of post-mortem pH decline, with the largest extent in LD and relatively higher ultimate pH in IS and SS. ST was the lightest ($P < 0.05$) muscle, while SS was the reddest ($P < 0.05$) muscle in this study. After aging and cooking, SM presented the highest ($P < 0.05$) loss of water and was the toughest ($P < 0.05$) muscle, while IS had the lowest ($P < 0.05$) cooking loss and was the most tender ($P < 0.05$) muscle. These data show that the different muscles varied in their on post-mortem biochemical and physical changes, and the gel results indicate that the methodology developed is suitable for observing fiber types in lamb muscles.

Keywords: lamb, longissimus dorsi, semimembranosus, semitendinosus, supraspinatus, infraspinatus, pH, muscle fiber type, color, tenderness, cooking loss

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Abbreviations

AK	Adenylate kinase
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
ATPase	Actomyosin adenosine triphosphatase
CK	Creatine kinase
DFD	Dark, firm, and dry
FG	Fast-twitch glycolytic
FOG	Fast-twitch oxidative glycolytic
IS	Infraspinatus
LD	Longissimus dorsi
LDH	Lactate dehydrogenase
MHC	Myosin heavy chain
NADH	Nicotinamide adenine dinucleotide
PCr	Phosphocreatine
pH_u	Ultimate pH
PSE	Pale, soft, and exudative
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SM	Semimembranosus
SO	Slow-twitch oxidative
SS	Supraspinatus
ST	Semitendinosus
WHC	Water-holding capacity

Chapter 1

Introduction

1.1 Background

Lamb, as a superior source of meat which is rich in essential nutrients like fats, amino acids, vitamins and minerals, has been widely consumed throughout the world. It is reported that 8,700,000 tons of lamb meat had been consumed during 2014, and the figure is projected to be 9,745,000 tons by 2025 (Colby, 2015). New Zealand, in particular, is the second largest exporter on lamb meat worldwide. In 2014, New Zealand exported more than 300,000 tons of lamb (Colby, 2015). With regard to the consumer's perception about lamb meat, the consumption of lamb meat is related to the lamb carcass quality (Beriain, Purroy, Treacher, & Bas, 2000). Therefore, maintaining lamb meat quality is significant for the lamb industry chain. Generally, lamb meat quality is associated with many aspects during the post-mortem period, including visual appearance such as color and other attributes like tenderness, juiciness, and water-holding capacity (Andrés et al., 2007; Gagaoua, Picard, & Monteils, 2018). These meat properties are related to many intrinsic (post-mortem metabolism and muscle fiber type) and extrinsic (sex, slaughter age and pre-slaughter stress) aspects of the muscle (Lawrie, 2006).

There have been numerous studies on the post-mortem physical and biochemical changes as animal muscle turns into meat in cattle and pigs (Veiseth, Shackelford, Wheeler, & Koohmaraie, 2001; Huidobro, Miguel, Onega & Blázquez, 2003). Considering the importance of lamb meat consumption globally, it is worth studying the post-mortem biochemical changes in lamb muscles and the result of meat quality properties.

1.2 Objective

The aim of this project was to analyze pH changes within five lamb muscles and correlate those to the physical and biochemical characteristics of the muscles. Secondly, to understand what drives the changes and determine the strength of the correlation in pH changes with meat quality properties.

1.3 Experimental Outline

Six lambs were slaughtered. Five muscles, three glycolytic, one oxidative, and one intermediate metabolism muscles, were examined from each lamb. The pH was measured directly on the carcasses at four times during the post-mortem period. The color of the meat was measured 24 hours after slaughter, and shear force and cooking loss were examined after 7 days aging. The fiber type of different muscles were visually analyzed by SDS-PAGE of the myosin heavy chain (MHC) isoforms.

Chapter 2

Literature Review

2.1 Muscle

2.1.1 Muscle Composition and Structure

Three basic kinds of muscles are identified in animals. These are smooth muscle, cardiac muscle, and skeletal muscle. Smooth muscle is located in the blood vessel walls, respiratory passage walls, uterine walls, and the lining of gastrointestine. It demonstrates slow but continuous contractile ability without conscious awareness, as it dominated by the autonomic nervous system. Likewise, cardiac muscle, which is controlled by the instrinsic nervous system particular with the heart, exists specifically for generating rhythmic contractions for the heart with involuntary thought. Skeletal muscle, controlled by the spinal cord nerves, forms the main volume of muscle in animal bodies and is the primary meat source in aninmal carcasses. As a movement organ in the body, it is connected with ligaments, tendons, or even bones in some conditions, providing movement and locomotion support for animal bodies, which will be the focus of the following sections (Kerth, 2013).

Muscle is a highly organised tissue that consists of individual cells, namely fibers, structured by connective tissue (Pearce, Rosenvold, Andersen, & Hopkins, 2011). According to Listrat et al. (2016), skeletal muscle is composed of around 90% muscle fibers and 10% connective tissues. They revealed that the connective tissue was divided into three levels of scale in the muscle. The endomysium surrounds each muscle fiber, while the perimysium was a collagen network, which surrounds the muscle fiber bundles. Finally, the epimysium located at the external surface, encircles the entire muscle (Listrat et al., 2016). In each fiber, the myofibril makes up most of the intracellular volume, and the sarcomeres within the myofibrils functions as the contractile unit of the muscle fiber (Listrat et al., 2016). Figure 2-1 displays the general organization of skeletal muscle.

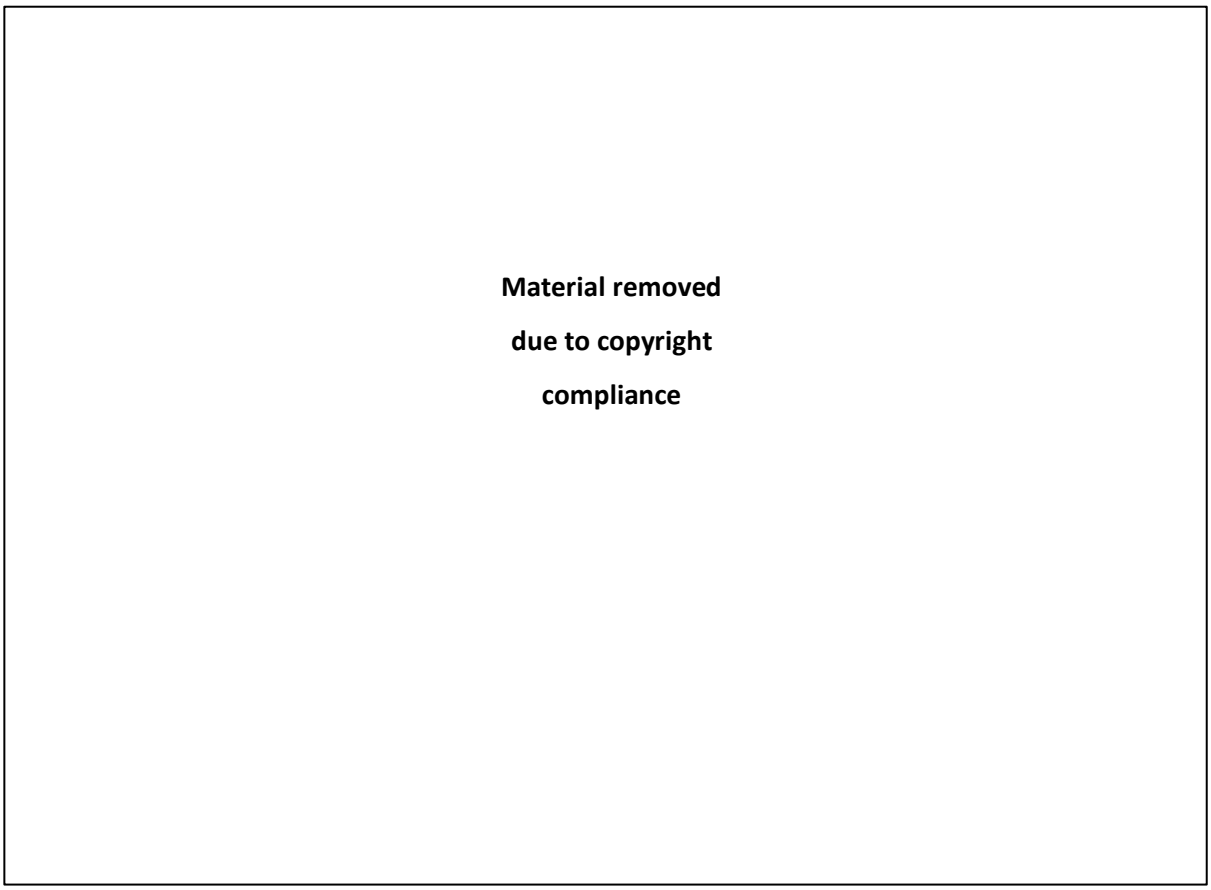


Figure 2-1. General organization of the muscle (Kerth, 2013)

The mass of livestock muscles makes up to 35% to 60% of the total body weight (Listrat et al.,2016). Muscle is composed of approximately 75% water, 19% protein, 2.5% intramuscular fat, and 3.5% other water-soluble compounds (Lawrie, 2006). Water, as the major component of the muscle, is held within the structure of the muscle and muscle fibers. The locations of water in skeletal muscle are shown in Figure 2-2. There is about 85% of water located within the protein-dense myofibrillar protein network, namely the intra-myofibrillar water, which is found in the myofibrils between the thick and thin filaments. The remaining water is outside the myofibrillar network, namely the extra-myofibrillar water that can be found in three places: in the sarcoplasm between myofibrils which is called inter-myofibrillar water, between muscle fibers or in the inter-fasicular space namely inter-fascicular water, and in the extra-fascicular between the muscle fasciculi that is called the extra-fascicular water (Pearce et al.,2011). According to Huff-Longergan & Lonergan (2005), water in the musle was classified into three types. First, some water in the muscle bonds tightly to protein, which is called bound water or protein-associated water, taking only a small proportion of total water in muscle and presenting strong resistance to freezing and heat. Another one is defined as entrapped or immobilized water, which is not bound to protein and liable to dry and freeze, making up the dominant part of the water in muscle. The last fraction of water in the muscle is known as free water that is easily mobilized and can help remove entrapped water from its original space sometimes (Huff-Longergan & Lonergan, 2005).

Figure 2-2. The structure and component of skeletal muscle and the locations of muscle water (Pearce et al., 2011)

In terms of protein in skeletal muscle, it is generally divided into three types on the basis of solubility, which are myofibrillar protein, sarcoplasmic protein, and stroma proteins (Kerth, 2013). Myofibrillar protein, soluble in salt solutions, comprises approximately 60% of the total muscle protein volume, which is the most abundant protein forming the myofibril (Xiong, 2018). This type of protein is primarily made up of actin and myosin, and is further divided into contractile protein and cytoskeletal protein (Xiong, 2018). It provides the main source of the muscle contractile activity (Favier, Benoit, & Freyssenet, 2008). The sarcoplasmic protein takes 35% of the total protein volume, and is soluble in water and low ionic strength solutions (Xiong, 2018). The last and least protein is the stroma protein, consisting of collagen, reticulin, and elastin. It is mainly located within the intersititial space of muscle fibers, and is soluble in acid or alkaline solutions (Xiong, 2018).

With a very small proportion, the intramuscular fat includes the lipids in adipocytes that located between the muscle fibers and between the muscle tissues, and also a small volume of lipid that can be found in the muscle cells, namely the intramyocellular lipid (Addison, Marcus, LaStayo, & Ryan, 2013).

2.1.2 Muscle Fiber Type

Skeletal muscle is made up of different types of fibers, each of which differs in their biophysical and biochemical properties, such as contraction speed, oxidative and glycolytic capacity, and myosin heavy chain (MHC) composition (Schiaffino ei al., 1989; Choe et al., 2008). Particularly, MHC is the motor protein of muscle thick filaments and classified into at least nine isoforms (Wells, Edwards, & Bernstein, 1996; Eddinger, 1998). MHC isoforms with different characters account for different muscle properties (Wells et al., 1996). Meanwhile, different fiber types of muscle result in different glycolytic capacity. Glycolytic capacity is generally defined as the maximum value of the ability that carbohydrate like

glycogen is transformed into lactate or pyruvate by a muscle cell (Mookerjee, Nicholls, & Brand, 2016). Based on the previous literature, there are several different methods for classifying the muscle fiber. Xiong (1994) stated that muscle contraction speed was considered as a more widely applied classification, which classified muscles as fast twitch or slow twitch. The fast-twitch muscle was defined as reacting more quickly when getting access to stimulation, and the color of this type of muscle was close to white (Xiong, 1994). On the contrary, the slow-twitch muscle reacted more slowly to stimulation and was close to red muscle regarding the physiological trait (Xiong, 1994).

Goldspink (2010) revealed that muscle fibers consisted of three types, which were slow twitch oxidative (SO or type Ⅰ), fast twitch oxidative (FOG, FR or type Ⅱa), and fast twitch glycolytic (FG, FF or type Ⅱb). This classification was associated with the biochemical characteristics of muscles, like MHC isoforms and activity of respiration (Goldspink, 2010). Bar & Pette claimed that SO, FOG and FG skeletal muscles contained MHC Ⅰ, MHC Ⅱa and MHC Ⅱb, respectively. According to Goldspink (2010), the SO fiber was abundant in mitochondria, but the ATP hydrolyzation by myofibrils of this muscle was slow, resulting in fatigue resistance. The FOG fiber and FG fibers were basically the same in terms of the biochemical properties, such as the high myosin ATPase specific activity, except that the FOG fiber was richer in mitochondria compared to FG fiber (Goldspink, 2010). The majority of skeletal muscles in animals are considered to constitute a mixture of various fiber types in different ratios (Xiong, 1994). This view has been further strengthened by a large quantity of literature. For instance, Chauhan et al. (2019) concluded that in most muscles of the adult animals, skeletal muscle fibers consist of four different types, which were Type Ⅰ (slow-oxidative muscle), Type Ⅱa (fast oxido-glycolytic muscle), fast glycolytic Ⅱx and Ⅱb. The longissimus dorsi in cattle was been shown to contain approximately 24.7% type Ⅱa fibers, 46% type Ⅱb fibers and 29.3% type Ⅰ fibers (Devine, Ellery, & Averill, 1984).

Another common scheme for muscle fiber types is based on the histochemical properties of fibers. According to Pette & Staron (1990), the muscle fibers could be differentiated by myofibrillar actomyosin adenosine triphosphatase (ATPase) activity and reference enzymes of anaerobic and aerobic energy metabolism. Initially, two myofibrillar actomyosin ATPase (mATPase)-based fiber types, namely type Ⅰ and type Ⅱ was distinguished by using the histochemical assay fot ATPase activity. It was shown that the histochemical difference in the activity of mATPase between type Ⅰ and type Ⅱ fibers was associated with the contractile properties in different fiber types. Also, the histochemical reactions for enzymes of aerobic oxidative metabolism, such as succinate dehydrogenase, cytochrome oxidase, and nicotinamide adenine dinucleotide (NADH) tetrazolium reductase, distinguished different muscle fibers (Pette & Staron, 1990). This resulted from differences in those enzyme activities, which was related to the different mitochondrial content (high, intermediate, low). These three levels of

mitochondrial content led to different aerobic oxidative potentials (Pette & Staron, 1990). These researchers concluded that by combining the mATPase-based histochemical and metabolic enzyme-based study, three major fiber types in animal muscles were recognised, which were slow-twitch oxidative (SO), fast-twitch oxidative glycolytic (FOG), and fast-twitch glycolytic (FG). In addition, Moody & Cassens (1968), who examined the histochemical properties on two porcine muscles, claimed that muscle fiber types were classified as *red* (oxidative activity), *intermediate* (oxidative and glycolytic activity), and *white* (glycolytic activity), using NADH-tetrazolium reductase or succinate dehydrogenase stains for red fibers while ATPase and glycogen phosphorylase stains for white fibers. Moreover, based on the histochemical properties, Peter et al. (1972) classfied muscle fibers into type Ⅰ, ⅡA, and ⅡB, presenting slow twitch oxidative, fast twitch oxidative glycolytic, and fast twitch glycolytic fibers, respectively. Shortafter, Salviati, Betto, & Betto (1982) added type ⅡC fiber on the basis of histochemical staining and peptide mapping, corresponding the intermediate fiber.

The last widely applied classification method was proposed by Ashmore (1974), who classfied muscle fibers into three groups, namely β-red, α-red, and α-white, on the basis of stain reactions. This technique identified β-red fibers as dark brown, α-red fibers as clear in the middle and surrounded by a blue ring, and α-white as clear. These three groups of fibers were corresponded with type Ⅰ, ⅡA, and ⅡB fibers, respectively. Likewise, Hunt & Hedrick (1977) identified muscle fibers into β-red, α-red, and α-white groups. They demonstrated that the β-red and α-red were oxidative in nature, while α-red and α-white were glycolytic. This was confirmed by Kirchofer, Calkins, & Gwartney (2002), who checked the fiber-type composition on thirty-eight muscles of beef chuck and round. According to this study, muscles were classfied as red, intermediate, or white based on the muscle fiber distribution. If the muscle had more than 40% of β-red fibers, this muscle would be identified as red, while if the muscle had more than 40% of α-white fibers, this muscle would be identified as white, and the rest of the muscles were considered as intermediate muscles (Kirchofer, Calkins, & Gwartney, 2002). Using this approach, the muscles they classified as red (> 40% β-red fibers) were regarded as oxidative, while the muscles identified as white (> 40% α-white fibers) were glycolytic. Those muscles that identified as intermediate were regarded as both oxidative and glycolytic. Based on the results of Ithurralde et al. (2015), who investigated histochemical fiber types in 16 lamb skeletal muscles, infraspinatus and supraspinatus were classified as representatives of slow-oxidative muscles, while semitendinosus and longissimus dorsi were fast-glycolytic muscles, and semimembranosus was classified as a typical intermediate muscle.

2.2 Post-mortem Metabolism in Muscle

Post-mortem metabolism is a complex period after slaughter when a series of energetic, physical and biochemical reactions occurs in the muscle, converting muscle to meat (Matarneh, England, Scheffler, & Gerrard, 2017). The slaughter process includes stunning, exsanguination, removing the fleece, and evisceration (Faustman, 1994). After that, two important features of the muscle are disrupted, which are the energy system and pH homeostasis.

2.2.1 Energy System in Post-mortem Metabolism

Adenosine triphosphate (ATP), as the link between the production and use of energy within skeletal muscle, can be generated by three main pathways: the phosphagen system, glycolysis, and oxidative phosphorylation. When the post-mortem metabolism begins, the phosphocreatine (PCr) plays the initial role in creating ATP catalyzed by the enzyme creatine kinase (CK), stabilizing the muscle ATP content at the early stage (Matarneh et al., 2017). However, PCr only works for a short period to maintain the muscle ATP. After that, two enzymes, adenosine monophosphate deaminase (AMPD) and adenylate kinase (AK), are activated to transfer ADP into ATP (Pearson, 2012). During the reaction of the phosphagen system, it is noticeable that the net production of H^+ is zero, while adenosine monophosphate (AMP) accumulates. According to England, Matarneh, Scheffler, Wachet, & Gerrard (2015), the growing AMP concentration resulted from reduced AMP deaminase activity increased the speed and extent of glycolysis.

Once the PCr concentration has declined, the muscle ATP is mainly produced through glycogenolysis and glycolysis. During glycogenolysis, glycogen (the storage form of carbohydrate) in the muscle is hydrolyzed to create ATP, as well as pyruvate and H^+. Since the animal is exsanguinated during slaughter, there is no oxygen supply for further metabolism, and so the muscle anaerobically transforms the pyruvate into lactate in a reaction catalyzed by lactate dehydrogenase (LDH). It was clarified by Matarneh et al. (2017) that although lactate concentration increases during glycolysis, it doesn't contribute to the pH decline. The decreasing pH is actually caused by the accumulation of H^+ in the muscle. Meanwhile, lactate is recognized as the indicator for the extent of post-mortem metabolism, as one H^+ is produced for each one lactate molecule (Matarneh et al., 2017).

As the last pathway for post-mortem energy generation, oxidative phosphorylation mainly occurs in the mitochondria. Mitochondria is a organelle in the cell that provides the place for tricarboxylic acid cycle (in the matrix) and electron transport (in the inner membrane) to synthesize energy (Pearson, 2012). This process shares the same initial reactions as glycolysis in that glycogen is degraded to pyruvate, before generating cellular energy through the tricarboxylic acid cycle in the first short period of post-mortem metabolism when the carcass has not been entirely exsanguinated and the oxygen is

still able to be carried in the blood (Lawrie, 2006). The oxidative capacity of mitochondria differs in different types of muscle, which impacts on the onset and extent of post-mortem anaerobic metabolism (Matarneh et al., 2017).

2.2.2 pH Change in Post-mortem Metabolism

During the post-mortem process, with the hydrolysis of ATP and glycogen, the muscle pH decreases from approximately 7.2 to the ultimate pH around 5.5 in most mammals (Matarneh et al., 2017). The post-mortem pH usually falls sharply during the early eight hours and then decreases slowly before stabilizing. The ultimate pH is reached when the muscle glycogen runs out or glycolytic enzymes are denatured and inactivated, normally at 24 ~ 48 hours post-mortem (Lawrie, 2006). Figure 2-3 shows the general trend of pH decline among common animals.

Figure 2-3. General trend of pH decline in longissimus dorsi among horse, ox, and pig (Pearson, 2012).

Based on Pearson (2012), factors including environment temperature, species, muscle fibers, and pre-slaughter stress are responsible for the variation in the rate of pH decline. Briefly, high temperature speeds up glycolysis and makes pH decrease quickly, while low temperature slows the rate of pH decline. The pH change also has a relationship with animal species. It is reported by Matarneh et al. (2017) that poultry muscle has the fastest pH decrease, followed by pig, and cattle, with lamb the slowest. This variation may result from the different amount of intracellular free Ca^{+} released from the mitochondria, because the Ca^{+} is able to increase the rate of glycolysis by activating phosphorylase kinase (the major enzyme in glycogenolysis) (Pearson, 2012; Matarneh et al., 2017). In addition, as mentioned in 2.1.2, different muscles fibers have different glycolytic capacities, which influence the rate of decrease in pH. Since there are fewer glycolytic enzymes in oxidative muscles, they demonstrate lower glycolytic capacity leading to a slower rate of glycolysis, compared to glycolytic muscles (Matarneh et al., 2017).

On the other hand, the ultimate pH (pH_u) which shows the extent of post-mortem metabolism is related to the quality of meat color, texture, and water-holding capacity. Przybylski & Hopkins (2015)

explained that the meat with low pH_u (normally < 5.4) appears to be pale, soft, and exudative (PSE), while high pH_u (≥ 6.0) results in dark, firm, and dry (DFD) meat. Figure 2-4 shows the rate and extent of post-mortem pH decline among normal and abnormal post-mortem metabolisms.

Figure 2-4. The rate and extent of post-mortem pH decline among normal and abnormal post-mortem metabolisms (Matarneh et al., 2017).

Research by Chauhan et al. (2019) has shown that muscle fiber type is one of the factors that impact on the pH_u of the muscle. They indicated that the glycolysis in oxidative muscles is terminated early compared with glycolytic muscles, leading to excessive residual glycogen and less lactate products, thus, the pH_u of the oxidative muscle is higher than the glycolytic muscle. Matarneh et al. (2017) summarized the factors including muscle glycogen concentration and loss of adenosine nucleotides that may determine the premature cessation of muscle glycolysis.

2.2.3 Aging

Generally, when the muscle reaches the ultimate pH, other chemical changes still proceed before the meat is cooked or futher processed, which is defined as aging or conditioning (Lawrie, 2006). Aging is a period between animal slaughter and meat consuming that usually takes place under refrigeration temperature (-1 to 4 ℃). Through this period, flavor, texture, and aroma of meat are changed, and the tenderness is improved (Matarneh et al., 2017). These chemical changes in aging process are associated with protein denaturation and proteolysis (Koohmaraie, 1994). According to Lawrie (2006), protein denaturation is a process that proteins are rearranged in the cells without hydrolysis of chemical bonds, resulting in molecular shape change and solubility decline. During proteolysis, proteins are broken and hydrolyzed into polypeptides or amino acids. Proteolysis results from the activity of proteolytic enzymes, including the calpain system, proteasome, cathepsins, and the caspase system (Koohmaraie, 1994).

2.3 Meat Quality Properties

The quality of meat has been considered as the predominant factor that influence consumers' preference and acceptability of lamb meat for consumption (Arsenos et al., 2002). Meat quality is associated with many extrisinc (such as slaughter age, animal weight, and sex) and intrisinc factors (such as muscle fiber type and post-mortem metabolism) of the muscle (Tougan et al., 2013). The appearance, tenderness and juiciness are the major attributes of meat quality, which are associated with the interactions of physical and chemical characteristics of meat (Andrés et al., 2007; Seideman, Cross, Smith, & Durland, 1984). In general, meat color is decided by the amount and state of myoglobin, muscle structure, and the capacity of muscle to absorb light (Seideman et al., 1984). Tenderness of meat is influenced by the amount and solubility of collagen, and the extent of proteolysis of myofibrils. The juiciness of cooked meat is related to the amount of intramuscular fat and the water-holding capacity of meat (Tshabalala, Strydom, Webb, & de Kock, 2003).

2.3.1 Color

The intensity of color is influenced by ante-mortem (species, sex, age, and stress of animals) and post-mortem (rate of pH decrease and ultimate pH) aspects (Seideman et al., 1984). According to Seideman et al. (1984), the color of lamb, as well as the myoglobin levels, is intermediate between beef (darkest in color with the highest myoglobin level) and pork (lightest in color with the lowest myoglobin level). In the muscle cell, myoglobin stores the oxygen that has been received from hemoglobin for further metabolism (Seideman et al., 1984). Myoglobin, a globular protein consisting a heme group surrounded by a globin moiety, constituting 95% of the total iron content when the animal is well-bled after slaughter so that hemoglobin (the primary pigmentary compound for meat) is almost removed entirely (Seideman et al., 1984). The difference in the color of myoglobin substantially results from the valence state of the iron atom (Fe) (Seideman et al., 1984). There are three principal and important forms of myoglobin determining the dynamic color reaction inside the body, which are reduced myoglobin (no oxygen metmyoglobin, Fe^{2+}), oxymyoglobin (oxygenation of myoglobin, Fe^{2+}), and metmyoglobin (oxidised myoglobin, Fe^{3+}). For fresh meat, the oxymyoglobin plays the most significant role, as it demonstrates the bright red color that meets consumer's demand (Lawrie, 2006). Also, the reduced myoglobin presents a purplish-red color, which is the main color form with the absence of oxygen, while the metmyoglobin causes a brown color for meat (Lawrie, 2006).

In addition, the rate and extent of pH decline and the morphology of muscle also impacts the intensity of meat color (Seideman et al., 1984). With a rapid post-mortem decrease and low ultimate pH, the meat appears to become paler in color. This is caused by the denaturation of the sarcoplasmic and myofibrillar proteins with a high carcass temperature, and the lactate accumulation that results from anaerobic metabolism of glucose and glycogen (Matarneh et al., 2017). Besides, the muscle fibrils are

open when the pH becomes low, thus, more light is scattered in the muscle, making the color of meat pale visually (Seideman et al., 1984). Also, the generation of metmyoglobin by oxidation of myoglobin is greater with the low ultimate pH, accounting for the paler and lighter color (Lawrie, 2006). For instance, when the ultimate pH is low and the rate of falling pH is fast, the meat of pig looks very pale, which is defined as PSE (pale, soft, and exudative) condition. On the contrary, with a high ultimate pH, meat appears to be dark in color. This situation results from insufficient glucose or glycogen after slaughter for glycolysis and the resistance to absorb light or oxygen as the muscle fibers are tightly connected (Seideman et al., 1984). Besides, since the oxygen can not diffuse in the muscle, oxymyoglobin, which is responsible for bright red color, cannot be generated from myoglobin (Lawrie, 2006). A common example of this condition is called DFD (dark, firm, and dry) in beef and pork.

Moreover, the meat color is associated with the water-holding and distribution in meat (Matarneh et al., 2017). For instance, more free extracellular water is presented in PSE meat, which results in a more open structure of meat so that more light is reflected, while in DFD meat, more intracellular water is found, increasing the chance of absorbing light in meat and causing a darker appearance (Matarneh et al., 2017).

2.3.2 Tenderness

Tenderness of meat is widely recognized as a complex combination of the elementary structure and the physiology of meat, and the structural element primarily influences the toughness of meat (Tornberg, 1996). Tenderness is related to a series of factors containing preslaughter factors (species and animal age) and post-slaughter factors (post-mortem glycolysis, proteolysis, aging and cooking). Obviously, species is the predominant factor that influence the tenderness of meat, as the concentrations of connective tissue, particularly collagen, in different animals varies a lot (Lawrie, 2006). Also, younger animals always have less collagen and this collagen is more digestible, resulting in more tender texture of meat (Zapata, Zerby, & Wick, 2009). The tenderness of meat is also associated with the rate and extent of post-mortem glycolysis. Lawrie (2006) indicated that the meat becomes soft when the post-mortem pH declines dramatically fast, because the denaturation of myofibrils makes them more insoluble and a high percentage of insoluble myofibrillar proteins is correlated with tenderness. As is mentioned in 2.2.2, high ultimate pH makes meat dry, firm, and dark (DFD) while low ultimate pH leads to pale, soft and exudative (PSE) condition. According to Tornberg (1996), an aging period at chilling temperature has a positive impact on meat texture. During aging, the calpain system for myofibrillar and cytoskeletal proteolysis is considered as the major protease that causes the meat post-mortem tenderisation. This involves in calpain-1 (μ-calpain), calpain-2 (m-calpain), and calpain 3 isoforms (Maltin, Balcerzak, Tilley, & Delday, 2003; Matarneh et al., 2017). In terms of cooking, based on Lawrie (2006), the collagen is transformed to gelatin during cooking, making the connective tissue more tender. This is affected by cooking time and temperature. Time

plays an important role in softening collagen while the temperature is crucial for toughening myofibrillar (Lawrie, 2006).

2.3.3 Water-holding Capacity and Cooking Loss

Defined by Honikel & Hamm (1994), water-holding capacity (WHC) is the ability of meat to retain its own or added water entirely or partially. The water content of meat plays an important role in determining the meat quality, especially tenderness and juiciness (Cheng & Sun, 2008). As is reviewed in 2.1.1, three types of water are classified in muscle cells, which are bound water, entrapped water and free water. The entrapped (immobilized) water takes up the main proportion of cellular water that is influenced by the post-mortem period (Huff-Lonergan & Lonergan, 2005). Various factors contribute to the different WHC consequences among animal muscles. Importantly, with the lactate concentration increases during the post-mortem metabolism, the muscle pH declines gradually. When the pH reaches the isoelectric point of the myosin, the net charge of the protein becomes zero, the positive and negative ion groups are attracted so that the water is not able to be held by the protein (Huff-Lonergan & Lonergan, 2005). This also restructures the myofibril and reduces the space in myofibril for binding water (Huff-Lonergan & Lonergan, 2005). Besides, the proteolysis during post-mortem period is also related to the WHC. According to Matarneh et al. (2017), the degradation of proteins leads to the loss of the intramyofibrillar water, as well as the loss of proteins connecting the myofibril and cell membrane, additionally causing the myofibril shrinkage and ultimately making the water slip away.

As the meat product is usually consumed after cooking, it is worth investigating the WHC of cooked meat, namely the cooking loss of the meat. During thermal cooking, proteins in meat are denatured, and the intra- and extra-cellular water are released (Honikel & Hamm, 1994). Cooking temperature is the key factor affecting cooking water loss, demonstrating a positive relationship between temperature and loss of water (Barbanti & Pasquini, 2005). Other factors including meat shape, size, and the cooking environment (in solution, air, or wrapping) also impact on the cooking loss of meat to some extent (Honikel & Hamm, 1994).

Chapter 3

Methods

3.1 Experimental Design

3.1.1 Materials

Five different muscles were chosen for this experiment. On the basis of muscle fiber types, these selected muscles consist of two fast-glycolytic muscles (semitendinosus and longissimus dorsi), two oxidative muscle (infraspinatus and supraspinatus), and one intermediate metabolism muscle (semimembranosus). Semitendinosus (ST) and semimembranosus (SM) are located in the hindlimbs of lamb and ST lies laterally to SM, while infraspinatus (IS) and supraspinatus (SS) are two adjacent muscles, situated in the forelimbs. Longissimus dorsi (LD) is the longest muscle in the loin, parallel to the spine. The diagram of muscle locations is shown in Figure 3-1, and more representative pictures of the five muscles are presented in Appendix A.1 and A.2. For each muscle, samples were taken from each of six lamb carcasses. Color, shear force, cooking loss, pH, muscle fiber type were measured on each sample.

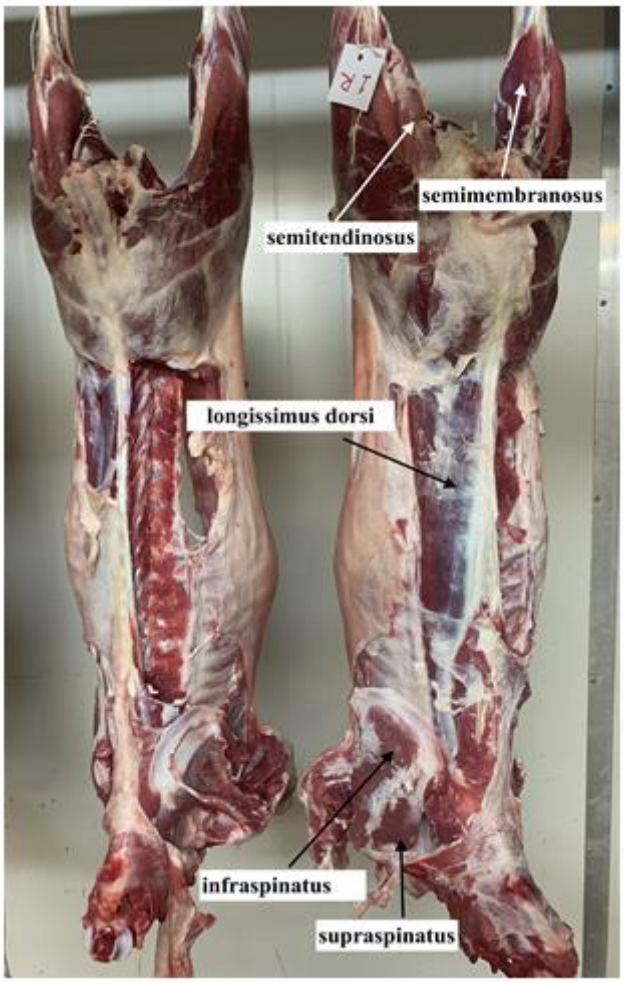


Figure 3-1. Diagram of the positions of five muscles in lamb carcass

3.1.2 Sampling

For the current experiment, five muscles were presented, and each muscle had six biological replicates. For each animal both side of the muscles were available (left and right side). The left side was used to study four hours post-mortem. The five muscles on this side were entirely excised at four hours, vaccum packed in aluminum foil vaccum-sealed bag individually and frozen at - 80 ℃ before biochemical analysis (fiber type). The other side of the carcass was used to measure the pH change and quality properties of 24 hours post-mortem. On this side, muscles were left in the chilling room (temperature at 4 ± 1 ℃) and pH was measured at this side on 4 different times. After the final measurement of the pH, muscles on this side of each carcass were entirely excised to measure the color. Then, these muscles were cut into two parts (small and the remainer). The small parts were vaccum packed in aluminum foil vaccum-sealed bags individually and frozen at - 80 ℃ before doing further biochemical analysis, while the large parts were aged at 4 ± 1℃ for seven days. Then cooking loss and shear force were measured. The table below shows the chemical analysis and the number of samples that were taken at different times after slaughter.

Table 3-1. The chemical analysis and the number of samples for different measurement at different times.

Time / Side	1.5 hours	4 hours	6 hours	8 hours	24 hours	7 days
Left		Fiber type, [5×6][1]				
Right	pH [5×6][1]		pH [5×6][1]	pH [5×6][1]	pH, [5×6][1]	Cooking loss, Shear force [5×6][1]

[1] means that five selected muscles were taken from six animals

3.2 Methods

3.2.1 pH and Temperature

pH and temperature were measured directly in the target muscles using a Hanna HI 9025 pH meter. This pH meter has an electrode for measuring pH and a metal probe for checking temperature, which is able to measure the pH and temperature value of a muscle at the same time by inserting the probes into it. The meter was calibrated prior to the measurement of six carcasses using pH 4.00 and pH 7.00 buffers at 37 ℃, and probes were cleaned by distilled water. The electrode for pH was inserted approximately 2 ~ 3 cm lateral to the midline of each muscle to make sure the measurements occurred near the center of the muscle, while the temperature probe was inserted about 2 cm away. The measurements of pH and temperature were taken in the same place for each time point.

3.2.2 Color

Surface color (lightness, L*; redness, a*; yellowness, b*) of raw meat samples were evaluated using a CR400 Chromameter (Konica Minolta, Japan). Color determinations were performed 60 minutes after removing muscles from carcasses. Color was checked on six different locations on every sample's surface. After measuring, the chroma (C) and hue angle (HA) were calculated using the following equations.

$$C = (a*^2 + b*^2)^{1/2}$$
$$HA = \tan^{-1}(b*/a*)$$

3.2.3 Cooking Loss

The cooking loss was determined as weight loss (%) after cooking each of the chilled muscles. Before cooking, meat samples were weighed. Then, these samples were cooked individually in plastic bags immersed in a water bath maintained at 80 ℃ until they attained an internal temperature of 75 ℃ as measured individually using Fluke type K temperature probes attached to Fluke 52 thermometer (Fluke Corp., Everett, WA). After that, samples were cooled at room temperature and patted dry with paper towels before weighing. Cooking loss percentage was calculated from the difference in the weight of raw and cooked samples, using the equation below.

$$Cooking\ loss\ (\%) = \frac{weight\ before\ cooking - weight\ after\ cooking}{weight\ before\ cooking} \times 100$$

3.2.4 Shear Force

Shear force was determined as per the method described by Chrystall & Devine (1991) using a MIRINZ tenderometer and was based on ten replicate measurements for each sample. After cooking meat samples as explained in 3.2.3, these samples were chilled overnight. The cooked samples were then cut into strips with a cross section area of 10 × 10 × 25 mm, parallel to the muscle fiber direction using a double-bladed scalpe with blades set 10 mm apart. The meat strips were placed onto the metal wedge of a MIRINZ tenderometer (AgResearch MIRINZ, Hamilton, New Zealand). The tenderness values were obtained by measuring the peak force required to cut across the meat strip, and mean force values (reported in Newton, N) were used in the statistical analysis. The mean reading of the MIRINZ tenderometer (kPa) were converted to shear force (kgF) and then to Newton (N) using the equation followed.

$$Shear\ force\ (N) = [(kPa\ \times 0.216) - 2.030] \times 9.8$$

3.2.5 Muscle Fiber Typing

Muscle fiber typing was followed by the method of Picard, Barboiron, Chadeyron, & Jurie (2011) with modifications. The proteins from 400 mg of muscle were extracted with 10 ml of the buffer containing 0.5M NaCl, 20mM sodium pyrophosphate, 50mM Tris, 1mM EDTA, and 1mM dithiothreitol (prepare freshly each day). Those samples were homogenized with a FJ200 high-speed dispersion homogenizer (Biaoben Model Corp., Shanghai) at 23,000*rpm for 30 seconds and centrifuged at 2,500*g for 10 minutes at 4 ℃. Diluting supernatants 1:1 v/v with glycerol. Next, the samples were suspended in 1:1 v/v in basic 2×Laemmli buffer (4% w/v SDS, 10% v/v β-mercaptoethanol, 20% v/v glycerol, 125mM Tris (pH 6.8) and 0.004% w/v bromophenol blue), incubated at room temperature for 10 min and then heated (70 ℃) for 10 min. The stacking gel and separating gel constituents were prepared from stock solutions as Table 3-2, and polymerisation was initiated with 0.05% v/v TEMED and 0.1% w/v ammonium persulphate. The gel volumes listed provide for three 0.75-mm-thick gels.

Table 3-2. SDS-PAGE gel mixtures and stock solutions

Stock solutions		Separating gel (ml)	Stacking gel (ml)
100% glycerol		4.5	3
30% acrylamide-bis[1] (50:1)		4.0005	1.333
Tris	1.5M pH 8.8	1.9995	
	0.5M pH6.7		1.4
1 M glycine		1.5	
100 mM EDTA pH 7.0			0.4
10% SDS		0.6	0.4
Distilled water		2.2425	3.362
TEMED[2]		0.005	0.005
10% ammonium persulfate		0.1	0.1

[1] Acrylamide-bis: acrylamide-N, N'-methylene-bis-acrylamide

[2] TEMED: N, N, N', N'-tetramethylethylenediamine

The running buffer consisted of 100mM Tris (base), 150mM glycine, 0.1% w/v SDS, and 0.07% v/v β-mercaptoethanol. 12 µl of each sample and 7 µl molecular marker (Precision Plus Protein™ Standards) were loaded onto 0.75-mm-thick gels set on a MiniProtean II Dual Slab Cell electrophoretic system (Bio-Rad). Three entire gel units were placed in the chilling room at 4 ℃ and electrophoresis was performed at a constant voltage of 70 V for 6 hours, 18 hours, and 30 hours, respectively. After migration, the gel was fixed in trichloracetic acid 15% (w/v) for 30 min and washed three times in distilled water for 5 minutes each time. Then the gel was stained in a solution of Gelcode for 1 hour at room temperature with gentle shaking on the rocker. After that, the stained gel was rinsed with distilled water and scanned.

3.3 Statistical Analysis

To evaluate the difference in the physical and biochemical characteristics among different muscles, collected data were analysed by analysis of variance (ANOVA) using the General Linear Model (GLM) procedure and a *posthoc* Tukey's Honestly Significantly Different (HSD) test ($P < 0.05$) by Minitab 18.

Chapter 4

Results

4.1 Carcass Weight

The heads, viscera and fleece of six lambs were removed immediately after slaughter, and the carcasses were weighed. Table 4-1 shows the individual carcass weight. The average weight of the six carcasses was 9.6 kilograms.

Table 4-1. Weights of six lamb carcasses

Carcass	01	02	03	04	05	06	Mean	SD[1]
Weight (kg)	9.90	8.56	9.38	10.60	10.30	8.86	9.6	0.74

[1] SD stands for standard deviation

4.2 pH Value and Temperature

4.2.1 pH Value

The means of pH values of the five muscles (six carcasses per muscle) in lamb are presented in table 4-2 with four testing times after slaughter (1.5 hour, 6 hours, 8 hours, and 24 hours). At 1.5 hours after slaughter, the ranking of pH values was as follow: LD > IS, SS > SM > ST. At 6 hours after slaughter, the ranking of pH values went as: IS > LD, SS > SM > ST. When it reached at 8 hours, the ranking changed as: IS, SS > LD > SM, ST. At 24 hours, the ranking of pH presented as follow: IS, SS > LD, SM, ST.

Table 4-2. Mean pH values of five muscles (n = 6 per muscle) in lamb at four times after slaugher

Muscle	pH			
	1.5 h	6 h	8 h	24 h
ST	6.20±0.14^{c}	6.02±0.21^{c}	5.90±0.15^{b}	5.68±0.05^{b}
SM	6.42±0.36^{bc}	6.20±0.27^{bc}	5.90±0.18^{b}	5.67±0.08^{b}
LD	6.93±0.19^{a}	6.51±0.23^{ab}	6.09±0.16^{ab}	5.81±0.15^{b}
IS	6.76±0.13^{ab}	6.60±0.09^{a}	6.30±0.11^{a}	6.05±0.11^{a}
SS	6.76±0.17^{ab}	6.46±0.12^{ab}	6.31±0.19^{a}	6.19±0.18^{a}

Data are means ± standard deviations;
Means that do not share a same letter are significantly different ($P < 0.05$) at the same post-slaughter time.

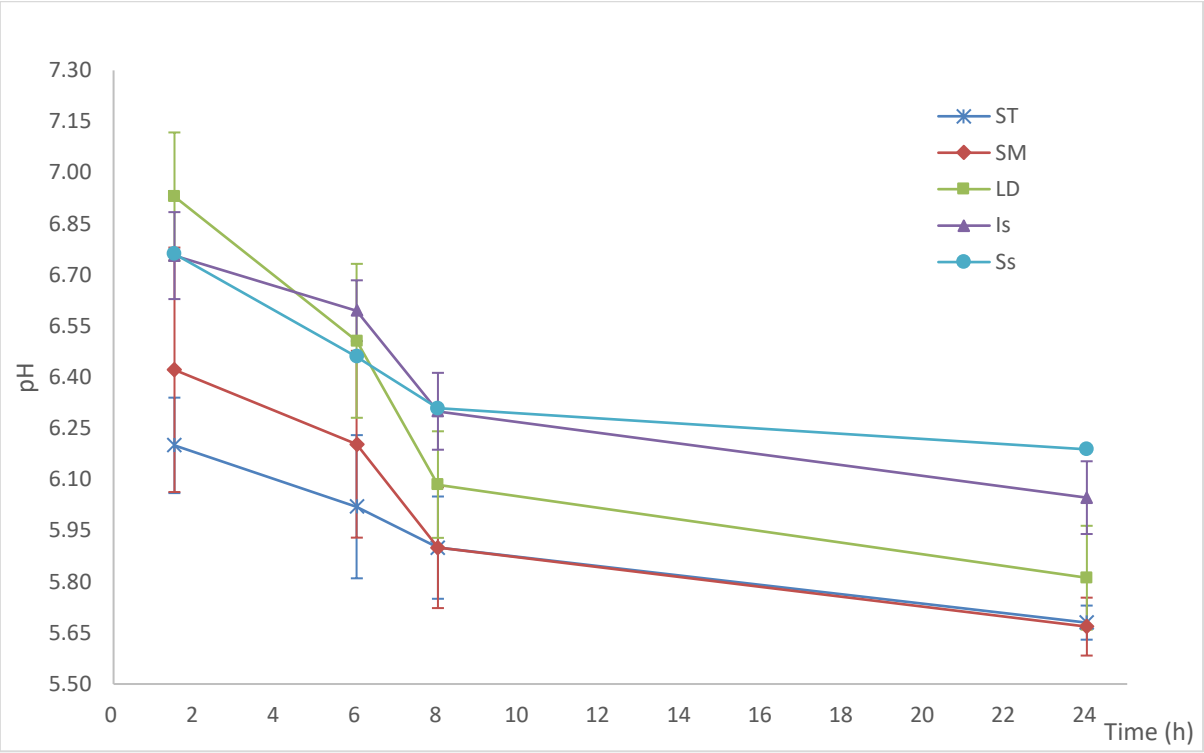


Figure 4-1. Kinetics of pH decline

4.2.2 Temperature

Over the 24 hours, post slaughter temperature declined from around 20 ℃ to 3 ℃. As shown in table 4-3, after 90 minutes, the temperature values of five muscles ranked as SM > IS, SS > ST, LD, while the temperatures among all the muscles were not significantly different at 6 hours after slaughter. Two hours later, the figures ranked as follow: SM > ST, LD, SS > IS. At 24 hours post-mortem, the ST muscle was the warmest, greater than SM and LD muscles, and IS and SS muscles were the coolest at last. By this stage, the temperatures of all muscles were 2.88±0.28 ℃.

Table 4-3. Mean temperature of five muscles (n = 6 per muscle) in lamb at four times after slaughter

Muscle	Temperature (℃)			
	1.5 h	6 h	8 h	24 h
ST	17.52±2.76^{b}	4.75±0.86^{a}	3.30±0.32^{ab}	3.20±0.35^{a}
SM	22.90±1.84^{a}	6.22±1.33^{a}	4.12±0.60^{a}	2.87±0.19^{ab}
LD	18.00±2.80^{b}	4.47±0.99^{a}	3.15±0.44^{ab}	2.87±0.10^{ab}
IS	20.95±2.09^{ab}	4.20±1.24^{a}	3.12±0.58^{b}	2.77±0.24^{b}
SS	20.52±1.82^{ab}	4.60±1.50^{a}	3.28±0.81^{ab}	2.68±0.25^{b}

Data are means ± standard deviations;
Means that do not share a same letter are significantly different ($P < 0.05$) at the same post-slaughter time.

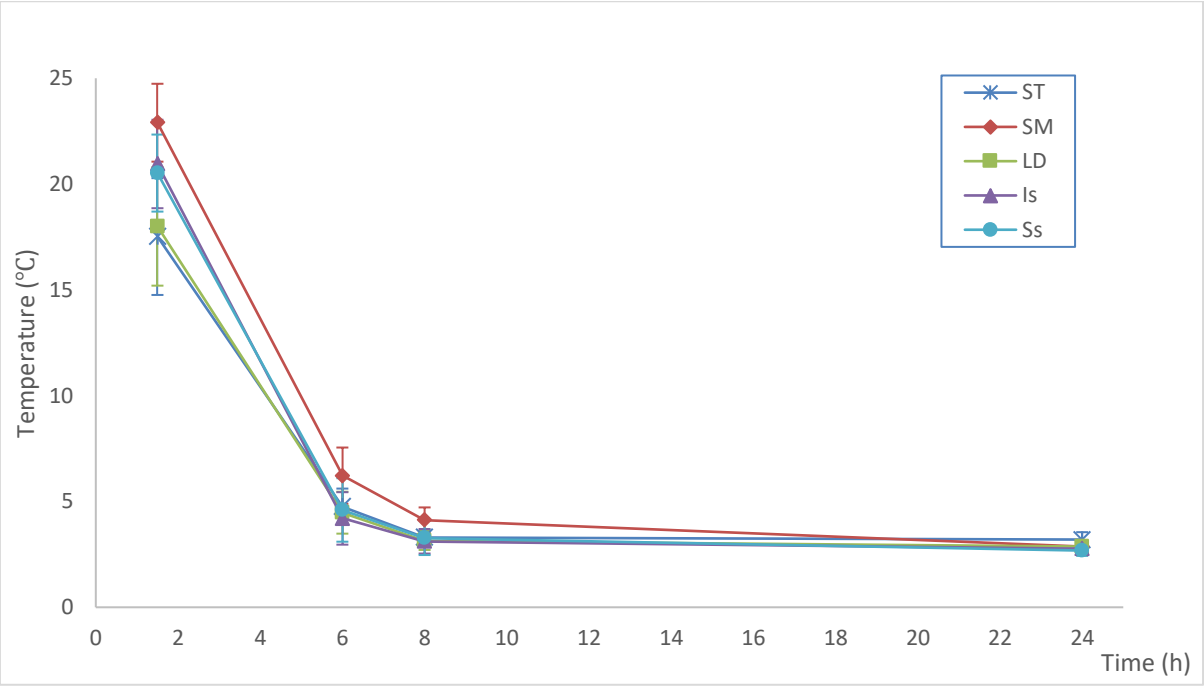


Figure 4-2. Kinetics of temperature decline

4.3 Muscle Fiber Type/Myosin Heavy Chain (MHC) isoform

Figure 4-3 presents the myosin heavy chain (MHC) isoforms (SDS-PAGE gel electrophoretogram) of five lamb muscles after 3 different gel running times. As the electrophoresis time increased, proteins in those muscles gradually went down and out of the gel, and the MHC isoforms became more clearly separated. At 6 hours, the MHC of each muscle were expressed on the gel but not clealy separated, and the bands of type Ⅰ fiber were slightly visible in SM, IS, and LD muscles. At 18 hours, the MHC-Ⅰ were clearly shown in all five muscles, while the MHC-Ⅱ isoforms were still integrated in one band. After 30 hours, the MHC-Ⅱ isoforms were entirely separated on the gel. In general, these lamb muscles contained type Ⅰ, Ⅱa, and Ⅱx myosin heavy chain (Sazili et al., 2005). For SM, IS, ST, and LD muscles, MHC-Ⅱa and -Ⅱx were more predominant than MHC-Ⅰ. On the contrary, the SS muscle contained predominantly MHC-Ⅰ. Compared to SM, ST, and LD muscles, the type Ⅰ band in IS was relatively thicker.

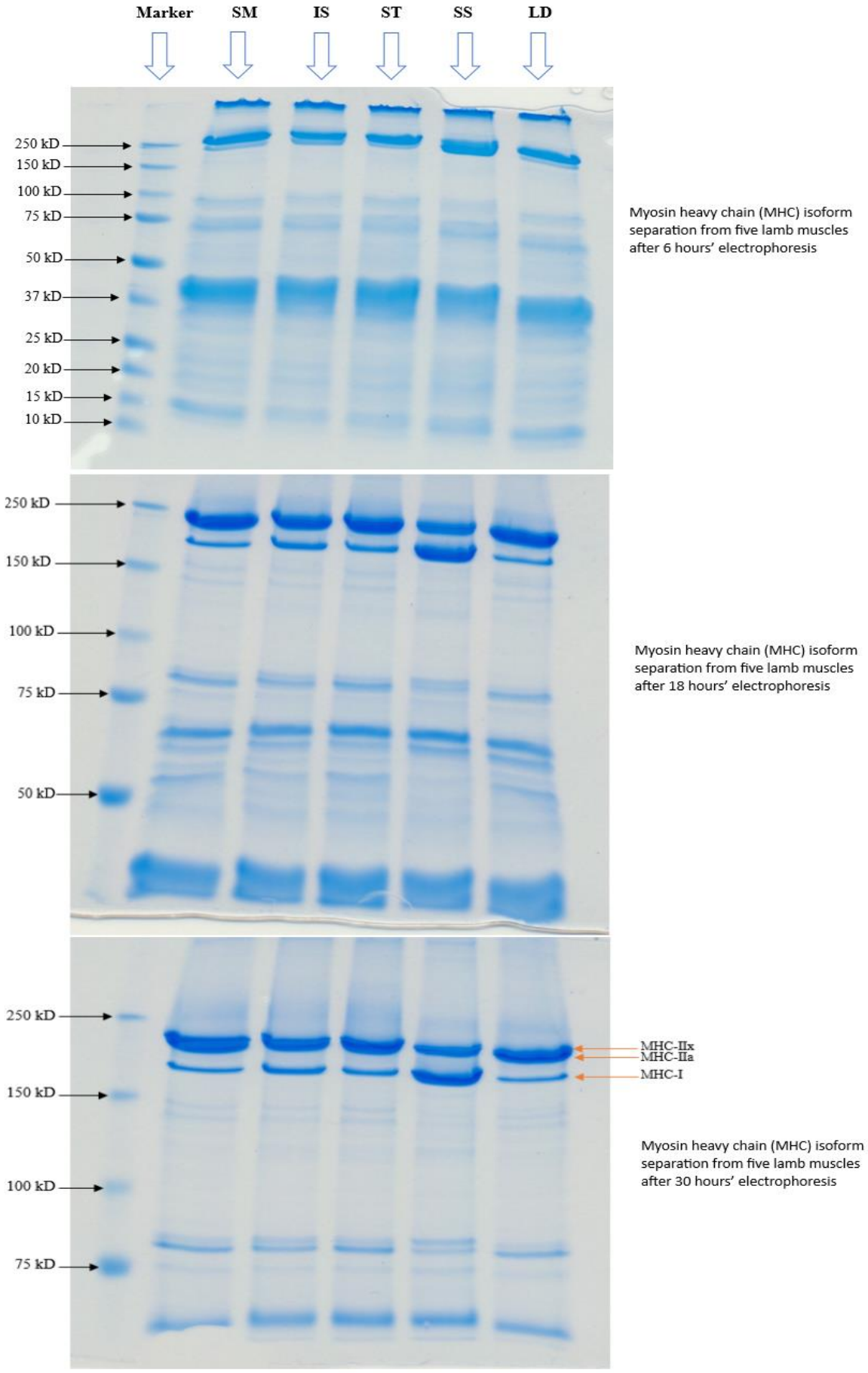


Figure 4-3. Myosin heavy chain (MHC) isoform separation from five lamb muscles after 6 hours, 18 hours, and 30 hours electrophoresis

4.4 Color

Table 4-4. Mean values of color parameters (L*, a*, b*, C, and HA) of five muscles (n = 6 per muscle) in lamb at 24 hours after slaughter

Muscle	Color Parameter				
	L*	a*	b*	C[1]	HA[2]
ST	41.97±2.80[a]	7.93±1.38[d]	2.50±1.12[a]	36.06±10.79[d]	0.31±0.16[a]
IS	38.32±3.12[b]	9.43±1.08[c]	1.45±1.28[b]	46.83±10.63[c]	0.15±0.13[b]
SS	36.77±2.87[b]	11.40±1.78[a]	1.35±1.20[b]	68.13±18.67[a]	0.12±0.12[b]
LD	34.04±2.13[c]	10.21±1.42[bc]	-0.49±2.04[c]	55.22±9.93[b]	-0.06±0.23[c]
SM	32.39±2.24[c]	10.35±0.85[b]	-0.48±1.46[c]	54.70±7.89[bc]	-0.05±0.15[c]

Data are means ± standard deviations;
Means that do not share a same letter are significantly different (P < 0.05) within the same column;
[1, 2] C stands for chroma and HA stands for hue angle.

The table 4-4 above shows the mean values of color parameters (L*, a*, b*, chroma, and hue angle) of five muscles (n = 6 per muscle) in lamb at 24 hours after slaughter. In general, these five muscles presented different characters in color. Regarding the lightness (L*) and yellowness (b*), the data of ST was higher (P < 0.05) than IS and SS, greater than LD and SM. Likewise, the calculated hue angle follows the same ranking. However, the results of redness (a*) differed a lot from L* and b*. SS had the highest a* among these muscles, while ST presented the lowest value. The redness of SM was relatively higher than IS. The results of a* corresponded the calculated chroma, which also shows the highest value in SS and the lowest in ST.

4.5 Cooking Loss

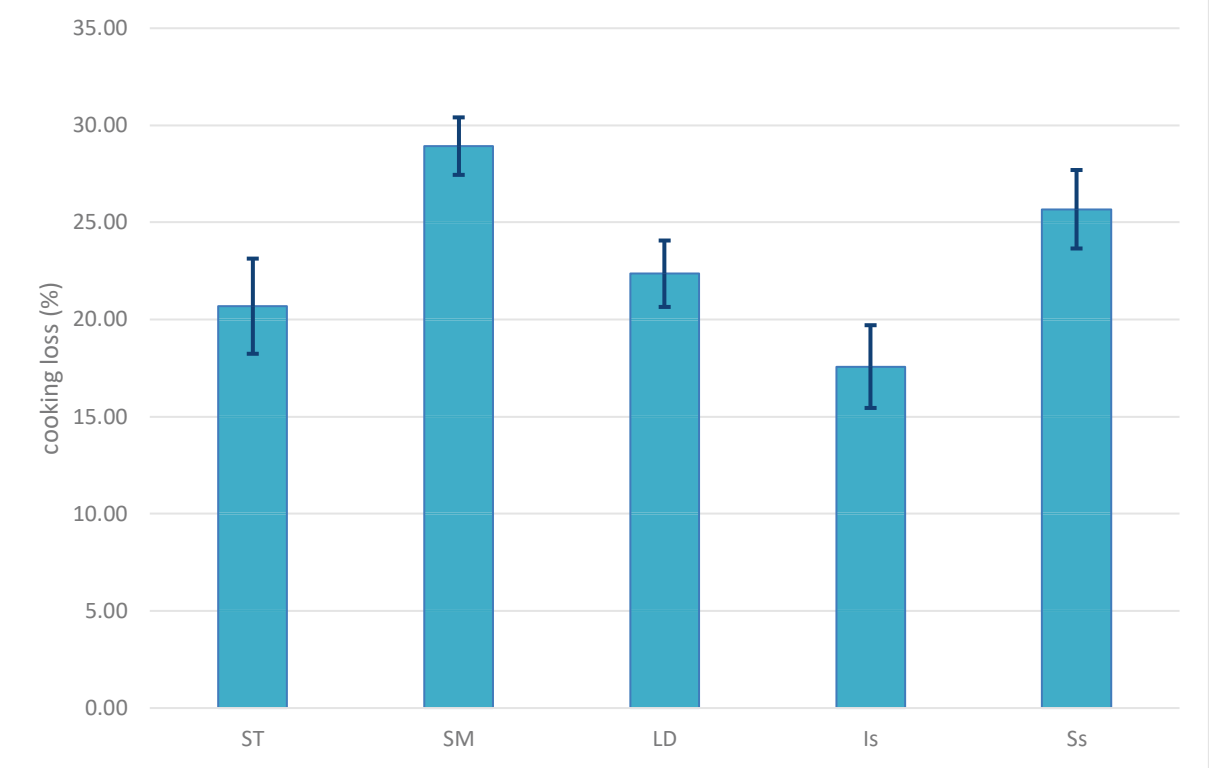
Table 4-5. Mean cooking loss (%) of five muscles (n = 6 per muscle) in lamb after aging for seven days

Muscle	IS	LD	SM	SS	ST
Cooking loss (%)	17.58±2.13[d]	22.36±1.71[bc]	28.93±1.48[a]	25.68±2.02[ab]	20.69±2.45[cd]

Data are means ± standard deviations;
Means that do not share a same letter are significantly different (P < 0.05).

Table 4-5 and figure 4-4 displays the cooking loss (%) of five muscles (n = 6 per muscle) in lamb after aging for seven days. As is clearly shown, the ranking of cooking loss (%) followed as: SM > SS > LD > ST > IS.

Figure 4-4. Mean cooking loss (%) with standard deviation of five muscles (n = 6 per muscle) in lamb after aging for seven days



4.6 Shear Force

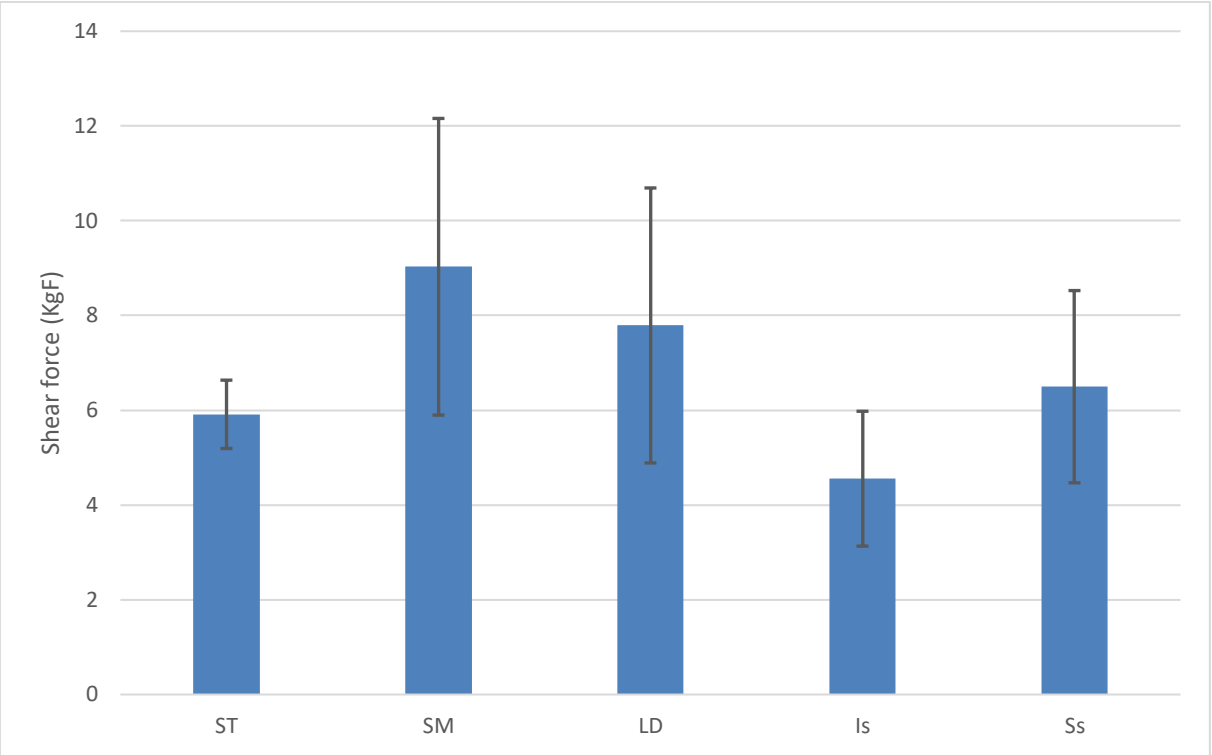
Table 4-6. Mean shear force (N and KgF) of five muscles (n = 6 per muscle) in lamb after aging for seven days and cooking

Muscle	IS	LD	SM	SS	ST
Shear Force (N)	44.64±13.95^{d}	76.34±28.44^{b}	88.47±30.68^{a}	63.67±19.88^{c}	57.96±7.07^{cd}
Shear Force (KgF)	4.56±1.42^{d}	7.79±2.90^{b}	9.03±3.13^{a}	6.50±2.03^{c}	5.91±0.72^{cd}

Data are means ± standard deviations;
Means that do not share a same letter are significantly different ($P < 0.05$).

Table 4-6 and figure 4-5 show the shear force of five lamb muscles (n = 6 per muscle) after aging for 7 days and cooking. The ranking of shear force (N) among fives muscles was SM > LD > SS > ST > IS.

Figure 4-5. Mean shear force (KgF) with standard deviation of five muscles (n = 6 per muscle) in lamb after aging for seven days



Chapter 5

Discussion

The results of the current study showed the variance of the biological and physical properties among five lamb muscles. The gel result clearly presented the different MHC isoforms in different types of muscles, which intrinsically led to the variance in post-mortem pH decline and meat quality characters. These muscles demonstrated different rate and extent of pH decline after slaughter. Likewise, they performed differently on color (lightness, redness, and yellowness), water-holding capacity (cooking loss), and tenderness (shear force). Various factors, including intrinsic and extrinsic aspects of muscles, are related to these differences. Details are going to be discussed in the following sections.

5.1 Muscle Fiber Type/Myosin Heavy Chain (MHC) Isoform

The separation of 4 MHC isoforms in cattle skeletal muscle using electrophoresis was described by Picard et al. (1999), which are type Ⅰ, Ⅱa, Ⅱx and Ⅱb, and was confirmed by Picard et al. (2011). However, according to a study done by Hemmings et al. (2009), when compared with cattle, only 3 main isoforms were observed in lamb muscles and no MHC-Ⅱb band was detected. The current study also found evidence for only 3 isoforms visible. They explained that the MHC-Ⅱb might co-migrated with the MHC-Ⅱx as previously shown in pig muscles that 4 MHC isoforms were expressed while only 3 of them were able to be separated. In addition, they stated that the MHC-Ⅱb isoform could be a minor isoform that was expressed at a less detectable level. A previous study has suggested that the minor isoform requires to be stained greater than 1% of the predominant band to be observed, when two isoforms co-migrated (Bottinelli, Canepari, Reggiani, & Stienen, 1994). Therefore, gel electrophoresis may not be the ideal method for detecting the MHC-Ⅱb isoform in lamb muscles.

During the process of this assay, we followed the methodology of Picard, Barboiron, Chadeyron, & Jurie (2011), who originally followed the method of Talmadge & Roy (1993), with modifications. Regarding the modifications, firstly we doubled the muscle weight and extraction buffer volume when extracting proteins, in order to get more proteins because the bands on the gel would be more visible with increasing protein concentration. Secondly, as the Polytron was not available in our laboratory and there was nothing written in their method about the speed and time, we chose the high-speed dispersion homogenizer (mentioned in 3.2.5) and applied the maximum speed to homogenize samples. Thirdly, Picard et al. (2011) added 0.01% w/v pyronin Y when preparing the Laemmli buffer. As the pyronin Y is a dye and more suitable for nucleic acids especially RNA (Li, Wu, & Gao, 2002), we used 0.004% w/v bromophenol blue for the protein dye. Moreover, in their gel systems, those researchers used separate lower and running buffer, but we were not able to do this. Thus, we applied

the recipe of their upper running buffer to run the gels. Lastly, as we hadn't quantified the protein, we alternatively chose to load 12 microliters of samples onto each well. Based on Sazili et al., (2005), bands of MHC isoforms were detected near 205 kDa, which was in accordance with the present study. By the end of the 30 hours' electrophoresis, type Ⅰ, Ⅱa, and Ⅱx MHC were separated clearly. This suggests that the major MHC isoforms of five lamb muscles were well-separated using this method.

Figure 5-1. Myosin heavy chain (MHC) isoform protein expression. (1) cattle cutaneus trunci muscle; (2) lamb SS muscle; (3) lamb ST muscle; (4) lamb longissimus thoracis (Hemmings et al., 2009)

According to Figure 4-3, the expression of MHC isoforms in ST, LD and SM muscles were similar and differed from the SS muscle. This result is in agreement with Hemmings et al. (2009), who observed the difference in MHC isoform expression between ST, LD and SS (Figure 5.1-1). As is described earlier, ST and LD muscles are typical fast-glycolytic muscles that are abundant in type Ⅱ fiber, while SS is a slow-oxidative muscle containing type Ⅰ fiber predominantly. However, in our result, SM and IS contained more type Ⅱ fibers than type Ⅰ, which are not in agreement with Ithurralde et al. (2015), who indicated that the IS muscle has more type Ⅰ fibers than type Ⅱ, while SM muscle is an intermediate (oxidative and glycolytic activity) muscle. They pointed out that this difference could be related to the different location (like superficial or deep in the muscle) when excising sample among various studies. They also revealed that breed and biotype of animals might contribute to this difference in fiber type composition.

5.2 pH decline

Normally, post-mortem muscle pH declines from around 7.2 initially to the ultimate pH between 5.5 and 5.7 (Apple et al., 1994). In the present study, the pH at 1.5 hours ranged from 6.20 to 6.93, which can be supported by McGeehin, Sheridan, & Butler (2001) who stated that the pH in the longissimus muscle of lamb carcass after 45 minutes ranges from 6.85 to 6.05. These researchers reported that the general pH decline in lamb muscles was influenced by carcass weight and ambient temperature to a lesser degree.

Figure 5-2. pH decline post-mortem in longissimus thoracis muscle for sheep (n = 17) and goats (n = 17) carcasses (Shijia et al., 2013).

In general, the trend of post-mortem pH decline in lamb has been widely reported in previous studies (Chrystall & Hagyard, 1976; Apple et al., 1994; Shijia et al., 2013). Figure 5-2 shows the post-mortem pH decline in longissimus thoracis muscle in lamb and goat. At the first 6 hours after slaughter, pH falls sharply from 6.50 (45 min) to around 5.80, and slightly decreases to around 5.70 at 24 hours. As expected, in the present study, the post-mortem pH among all five muscles roughly declined similarly with the curvilinear trend. Overall, the rate of pH decline for all of the muscles was greater for the first 8 hours and slowed down to 24 hours. It is very noticeable in the kinetics of pH decline (Figure 4-1) that the LD muscle had the fastest rate and largest extent of pH fall among all five muscles. Nagaraj, Anilakumar, & Santhanham (2005) indicated that the variation in the proportion between individual fibers of specific types in a muscle was responsible for the rate of pH decline.

It should be noted that the animals in the present study were not electrically stimulated when slaughtered. Generally, electrical stimulation is applied to accelerate the aging and rigor rate of animals (Chrystall & Hagyard, 1976). Chrystall & Hagyard (1976) compared the post-mortem pH decline in unstimulated LD muscle with the stimulated LD muscle, and suggested that electrical stimulation resulted in great difference in the rate of pH decline. Figure 5-3 shows the result of their study. At around 1 hour after slaughter, the pH value of unstimulated was higher than that of stimulated muscle, with the number at nearly 7.0. This corresponded our result, with the pH value of LD at 6.93 at 1.5 hour post-mortem.

Figure 5-3. pH decline in LD muscle of stimulated (——) and unstimulated (------)animals (Chrystall & Hagyard, 1976)

To be more specific, Apple, Unruh, Minton, & Bartlett (1993) measured the 24 hours post-mortem pH of lamb SM and IS muscles, and the average values for them were 5.60 and 5.95, respectively. The values in the present study were higher than those. Likewise, for ST and SS muscles, the results of pH at 24 hours were higher than that in the experiment done by Gault, Gordon, & Tolland (2005). In addition, Wheeler & Koohmaraie (1994), who checked the pH for longissimus muscle in lamb, reported the mean pH value at 24 hours for this muscle was around 5.81, which was the same compared to the current study. However, pH values for 1.5 hour, 6 hours and 8 hours were greater than previous studies. Kahraman et al., (2014) reported the pH at 1 hour after slaughter for LD was 6.77, which was lower than pH at 1.5 hour (6.93) in the present study. According to Apple et al. (1994); Wheeler & Koohmaraie (1994); Veiseth, Shackelfor, Wheeler, & Koohmaraie (2004), pH of lamb longissimus muscle at 6 hours was approximately between 6.1 to 6.3, while the pH value in present study was 6.51. Also, for the pH at 8 hours, the value in present study (6.09) was higher than the study done by Kahraman et al., (2014) , who mearsured the value for 5.96. Slaughter weight may explain the higher pH results. Caneque et al., (2001) found a significant difference in pH values between animals with different slaughter weight groups, with the pH in lower weight group higher than that in the higher weight group. In the present study, the average carcass weight was 9.6 kg, which was lighter compared to previous studies (normally with the slaughter weight at around 20~40 kg). Besides, in the present study, the ultimate pH of LD muscle (5.81) was lower than IS and SS muscles that were both above 6.0. This result is in accordance with Sitthigripong, Sethakul, & Chaosap (2013). They found the highest glycogen content in LD compared to IS and SS, and clarified that the variation of glycogen content

among muscle types could explain the different pH_{24h}, because glycogen content was associated with muscle glycolysis that would affect the ultimate pH. Meanwhile, fiber type is also responsible for the high ultimate pH values in IS and SS muscles. It is explained by Ithurralde et al. (2015) that IS and SS are chief representatives of slow-oxidative muscles, and slower and more oxidative muscles often result in higher ultimate pH.

5.3 Color

Realini et al. (2013) stated that meat quality properties like color, water-holding capacity and tenderness vary greatly among muscles. This is associated with the metabolic and contractile properties of muscles and muscle fiber type which is mainly responsible for the variation. Based on Sirin et al., (2017), meat color was associated with muscle fiber characteristics. They observed positive correlations between lightness, redness and type Ⅰ fiber number in LD and ST, respectively. Meanwhile, they found that for some lamb species, lightness value increased with the rising numbers and size of type ⅡA and ⅡB fibers.

Meat color is also related to animal carcass weight. Martinez-Cerezo et al. (2004) stated that the suckling lamb meat was lighter, and lightness declined with the increasing carcass weight. In terms of redness, they found meat from heaviest was redder. For yellowness, meat from younger animal and lighter weight presented more yellow.

On the basis of Calnan, Jacob, Pethick, & Gardner (2017), lightness (L*) of the chromameter stands for dark to light on the scale of 0 to 100, while redness (a*) and yellowness (b*) represent green to red and blue to yellow respectively, on the scale of -60 to 60. In the present study, ST was the lightest muscle with the highest L value but the lowest a* value, while SS was the reddest muscle with the highest a* value. This is in agreement with Ithurralde et al. (2015), who clarified that the fast-glycolytic muscle is normally associated with brighter meat, while the slow-oxidative muscle results in redder meat. It is claimed by Neethling, Suman, Sigge, Hoffman, & Hunt. (2017) that the acidity of meat is one of the most important factors influencing meat color. Specifically, the mean L* value of longissimus thoracis was reported at 34.9 in the study of Calnan et al. (2017), which is similar to our result (34.04). However, in comparison with the results of Vergara, Molina & Gallego (1999), who measured the color of LD muscle, and Tschirhart-Horlscher, Baird, King, McKenna, & Savell (2006) who also studied all the five muscles in our project, all the color values (L*, a*, and b*) in the present study were lower than their results.

Chroma is a value for indicating the saturation or vividness of color, and it follows the same trend as redness (a*) (Gao et al., 2013), which is in accordance with the present study. Meanwhile, hue angle (HA) is an indicator for discoloration and displays a more practical view on browning rather than single

color coordinates (Gao et al., 2013). According to Gao et al. (2013), meat with lower HA was considered as the color stable meat. Therefore, in the present study, ST with the greatest HA value was the least color stable muscle, while LD and SM were the most color stable muscles among all five muscles.

5.4 Cooking Loss

In the present study, the lowest proportion of cooking loss was identified in the IS muscle, in comparison with the LD, SM, and SS muscles. This result corresponded with Qurni, Abdullah, Olusesan & Malar (2011) who investigated muscle properties after post-mortem and aging on 9 types of lamb muscles. They refered that the IS muscle displayed the lowest ($P < 0.05$) cooking loss while the SM muscle presented the highest percentage. Meanwhile, they also revealed that there was no significant difference in cooking loss between SM and SS, which corresponds the present result. The result of cooking loss reveals the water-holding capacity of cooked meat. During the post-mortem aging, the muscle structure becomes looser because myofibrilar and cytoskeletal proteins are degraded (Huff-Lonergan & Lonergan, 2005). Later after thermal cooking, proteins in meat are denatured, resulting in the release of intra- and extra-cellular water (Honikel & Hamm, 1994).

However, when compared with Qurni et al. (2011), the values of cooking loss for all five muscles in the present study were slightly lower. This might be related to the cooking temperature. As is reviewed in 2.3.3, cooking temperature is the key factor affecting cooking water loss with a positive correlation (Barbanti & Pasquini, 2005). Higher cooking temperature results in more loss of water. In their study, they cooked meat samples until they attained the temperature at 75 ℃, which was higher than our cooking temperature. Besides, meat shape and size also impact on the cooking loss (Honikel & Hamm, 1994). In the present study, the whole muscles were cooked without attempting to make the size and weight uniform. In the study of Oillic, Lemoine, Groa, & Kondjoyan (2011), water decreased more slowly in larger samples within the same time. Moreover, MHC isoforms can influence the water-holding capacity (WHC) of lamb meat intrinsically. Fast-glycolytic muscles are normally related to lower WHC, which gives an explanation for the relatively lower WHC in LD and ST muscles.

In addition, during the aging period, drip loss occurs due to the post-mortem metabolism in the muscle. The myofibrillar water is expelled by the shrinkage of the myofibrils and move through the sarcoplasmic membrane, resulting in the accumulation of water in the extracellular space. The gap, namely the “drip channel”, is formed by the separation of muscle cells and fiber bundles and gives a way for water to reach the meat surface (Torres Filho, Cazedey, Fontes, Ramos, & Ramos, 2017). Before cooking, there have been drip loss in each muscle during aging for 7 days in the present study. The amount of drip loss influences meat quality properties, as the more drip loss of the muscle during aging, the less water expressed during cooking (Torres Filho et al., 2017). However, since we didn’t

quantify the amount of drip loss, we are not able to demonstrate the correlations between the drip loss and cooking loss among these muscles.

5.5 Tenderness

Based on Bickerstaffe, Bekhit, Robertson, Roberts, & Geesink (2001), relationship between shear force (KgF) results using the MIRINZ tenderometer and meat tenderness as assessed by consumers was as follows: shear force < 4.9, very tender; 5.0 < shear force < 7.9, tender; 8.0 < shear force < 10.9, acceptable; 11.0 < shear force < 14.9, tough; shear force ≥ 15, very tough. Therefore, in the present study, the IS muscle was very tender, and ST, LD and SS were tender, while SM was acceptable. Overall, all of these muscles were relatively tender on the basis of this classification scheme above. This can be explained by the younger animal age and lower slaughter weight. Young & Braggins (1993) reported that lamb meat tenderness was correlated with the collagen solubility that decreased largely with growing animal age.

After aging and cooking, SM was the toughest muscle that with the highest shear force among all the muscles, followed by LD. IS was presented to be the most tender muscle, with the lowest shear force. In general, the tenderness result is in accordance with a previous study (Qurni et al., 2011). They observed the highest shear force values in SM muscle while the lowest in IS. Also, Morgan et al. (1991) observed the highest shear force values in beef steaks from the top round, and the SM muscle is situated in the round. Besides, Sullivan & Calkins (2011) found IS was the most tender muscle among all these five muscles in beef. Likewise, in an experiment on beef muscles conducted by Rodriguez et al. (2013), a significantly lower value ($P < 0.05$) of shear force in ST was found in comparison with the longissimus muscle. There was no significant difference in shear force between ST and IS according to their study. The difference in tenderness between LD and ST muscles is explained by Kemp & Parr (2012) that the tenderness of LD is mainly influenced by the proteolytic capacity, as there is more chromatin condensation and apoptotic bodies observed in LD muscle that shows more apoptotic-mediated proteolysis occurs. However, Tschirhart-Horlscher et al. (2006) found that LD was the most tender muscle with the lowest shear force that was slight lower than IS, and Sullivan & Calkins (2011) reported LD was the toughest while SM was the second most tender muscle among all these five muscles. Rhee, Wheeler, Shackelford, & Koohmaraie. (2004) illustrated that LD was the most variable in shear force, followed by SM. It is fact that among various experiments the LD muscle is variable in tenderness in comparison with other muscles, thus, if it's relatively tender it would be the most tender muscle and if it's relatively tough it would be the toughest one, especially when the object number is small, as other muscles usually change little in tenderness compared with LD (Rhee et al., 2004).

The tenderness values of all five muscles in our study were higher than in some previous studies (Qurni et al., 2011; Sitthigripong et al., 2013). In the study of Qurni et al. (2011), the Warner-Bratzler shear force (KgF) values of five muscles were 4.1 (IS), 4.8 (LD), 5.1 (SM), 4.7 (SS), and 4.5 (ST). Likewise, Sitthigripong et al. (2013) reported the shear force (KgF) of IS, LD, and SS were 3.29, 4.00, and 4.02, repectively, using an instron Universal Testing Machine. The differences in tenderness among studies could result from the inconsistency of the testing machine. Meanwhile, different cooking method, aging time, and sample locations among experiments could cause great variation in the tenderness rank of meat (Rhee et al., 2004). Also, the concentration of intramuscular lipid is associated with the shear force with a negative correlation, and intramuscular lipid increases with decreasing moisture content during cooking (Okeudo & Moss, 2004).

Chapter 6

Conclusion

In this study, we investigated the variation of post-mortem metabolism and meat quality properties in five lamb muscles, namely ST, SM, LD, IS, and SS muscles. Overall, these muscles varied in their biological and physical characters. For the muscle fiber typing, SM, ST, and LD contained predominantly MHC-ⅡA and ⅡX, while SS was more abundant in MHC-Ⅰ isoform, and IS presented more MHC-Ⅰ fiber than SM, ST, and LD, but less than SS. This variation intrinsincally led to differences in other properties. During the 24 hours post-mortem, temperature of all muscles fell and reached nearly 3 ℃. In terms of pH, all the muscles followed the normal trend of post-mortem pH decline, with the largest extent in LD muscle and relatively higher ultimate pH in IS and SS muscles.

ST was recognized as the lightest color muscle, while SS was the reddest muscle in this study. After aging and cooking, SM presented the highest loss of water and was the toughest muscle, while IS had the lowest cooking loss and was the most tender muscle. All these muscles were classified as not tough.

Moreover, during the process of electrophoresis, we modified the methodology to adapt in to our laboratory. The gel results showed clear bands of MHC isoforms in all five muscles. Therefore, we considered that the method we developed could successfully visualize the fiber types of lamb muscles.

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Appendix A

Pictures of Lamb Muscles and Carcasses

A.1 Photo of slaughtered animals and muscle positions

A.2 Representative pictures of five lamb muscles 24 hours after slaughter


infraspinatus
supraspinatus
longissimus dorsi

Appendix B

Tables of Raw Data

B.1 pH and temperature values of five lamb muscles after slaughter

Carcass	Muscle	pH (1.5h)	temp (1.5h) ℃	pH (6h)	temp (6h) ℃	pH (8h)	temp (8h) ℃	pH (24h)	temp (24h) ℃
1	ST	6.27	13.6	5.88	3.8	5.74	3.6	5.67	3.8
1	SM	6.65	22.8	6.38	4.3	5.88	3.5	5.74	2.9
1	LD	6.93	15.3	6.12	3.5	5.84	2.8	5.85	2.9
1	IS	6.72	18	6.69	3.1	6.16	2.8	6.13	3.2
1	SS	7.04	20.2	6.54	3.6	6.2	2.7	6.22	2.7
2	ST	6.33	14.7	6.19	4	5.98	2.7	5.78	3.4
2	SM	6.85	20.8	6.47	4.9	6.03	3.3	5.8	3
2	LD	7.28	14.9	6.52	3.2	6.16	2.6	6.11	2.7
2	IS	6.87	19.1	6.63	3.7	6.2	2.6	6.17	2.8
2	SS	6.89	19.2	6.62	3.7	6.5	2.8	6.43	3
3	ST	6.19	20.5	5.76	4.2	5.7	3.3	5.67	3.1
3	SM	6.44	25.4	5.82	6.5	5.63	4.1	5.64	2.5
3	LD	6.74	21.2	6.34	4.3	5.93	2.9	5.85	2.9
3	IS	6.82	22.8	6.44	4.3	6.29	3.2	6.12	2.7
3	SS	6.73	21.6	6.44	4.9	6.57	3.5	6.36	2.8
4	ST	5.92	18.2	6.28	5.1	6.07	3.4	5.66	3.1
4	SM	5.82	21	6.4	7.3	5.84	4.5	5.65	2.9
4	LD	6.71	16.3	6.69	5.8	6.11	3.7	5.69	3
4	IS	6.58	20.6	6.66	6.3	6.43	4.1	5.93	2.8
4	SS	6.61	20.2	6.42	7.1	6.12	4.6	6.03	2.8
5	ST	6.22	18.4	6.12	5.7	5.98	3.5	5.63	2.9
5	SM	6.53	22.9	6.24	7.6	6.15	4.8	5.58	3
5	LD	7	20	6.81	5.1	6.31	3.5	5.67	2.9
5	IS	6.9	23.2	6.59	4.8	6.43	3.4	5.93	2.6
5	SS	6.59	23.5	6.48	5.3	6.29	3.7	6.11	2.5
6	ST	6.24	19.7	5.89	5.7	5.91	3.3	5.65	2.9
6	SM	6.24	24.5	5.91	6.7	5.87	4.5	5.6	2.9
6	LD	6.92	20.3	6.56	4.9	6.16	3.4	5.7	2.8
6	IS	6.65	22	6.56	3	6.29	2.6	6	2.5
6	SS	6.71	18.4	6.26	3	6.17	2.4	5.98	2.3

B.2 Values of color in five lamb muscles at 24 hours after slaughter (6~7 instrumental replicates for each muscle sample)

muscle	carcass	L	a	b	muscle	carcass	L	a	b
ST	1	41.48	8.62	2.11	SM	1	31.97	9.97	-0.6
		39.19	10.4	3.02			31.83	9.25	-1.15
		38.5	9.81	2.45			34.52	10.2	-1.31
		42	8.04	1.84			33.8	10.49	-0.18
		42.23	7.27	1.96			32.23	10.44	-0.63
		39.09	8.27	1.73			32.45	9.59	-0.39
	2	38.04	8.79	0.96		2	33.8	10.99	0.92
		43.39	7.94	2.77			34.14	9.89	-0.96
		40.77	8.2	2.6			29.58	10.41	-2.87
		39.93	9.24	3.12			30.67	9.75	-2.73
		39.23	9.87	3.45			31.33	11.18	-0.33
		42.09	8.36	3.15			33.02	11.76	0.59
	3	49.14	4.94	3.89		3	36.04	9.77	1.28
		47.62	5.42	3.39			34.98	10.12	1.4
		48.71	4.98	3.67			37.04	9.94	2.79
		45.49	5.74	1.91			35.12	10.34	1.21
		45.5	6.22	3.58			37.47	10.01	2.67
		44.57	6.8	3.56			35.47	10.15	0.99
	4	39.77	9.24	2.32		4	30.9	9.49	-1.51
		40.48	8.02	-0.23			31.51	11.87	-0.16
		41.59	8.42	1.73			31.57	11.05	-0.23
		39.46	9.3	3.15			30.3	10.94	-1.3
		43.52	7.89	2.78			34.24	7.77	-3.25
		42.6	9.35	3.02			30.41	9.84	-1.73
	5	42.38	7.15	1.47		5	32.91	9.32	-1.58
		42.56	7.23	3.1			30.38	11.54	-0.46
		42.35	6.86	2.37			30.84	9.9	-1.95
		42.63	7.08	2.77			30.71	10.41	-1.52
		42.58	6.56	2.34			32.2	10.44	-0.25
		44.45	7.52	-0.45			31.16	11.55	-0.0§
	6	38.52	8.69	0.89		6	30.57	10.37	-0.55
		39.89	8.63	3.4			31.52	11.59	-0.18
		40.98	9.16	4.48			31.22	10.3	0.15
		38.58	9.42	3.06			26.91	10.35	-2.91
		40.73	7.22	0.87			30.37	9.9	-1.05
		40.72	8.64	3.74			32.98	11.76	1.03

muscle	carcass	L	a	b	muscle	carcass	L	a	b
IS	1	36.63	10.9	1.52	SS	1	37.76	12.12	2.13
		37.21	10.24	1.37			40.55	10.72	2.96
		36.8	10.29	1.2			36.06	12.17	1.42
		41.05	8.41	1.23			37.65	11.95	2.01
		42.43	7.54	2.7			40.19	7.31	-0.95
		37.94	9.12	0.05			36.89	8.99	-2.28
	2	35.03	9.21	0.18			36.26	9.18	-1.86
		35.53	10.64	0.59		2	40.52	8.7	1.66
		33.67	10.89	0.51			33.71	11.09	0.39
		35.81	10.03	1.05			34.2	10.12	0.32
		36.97	8.65	0.61			32.66	13.08	0.82
		34.94	10.84	0.23			32.76	12.71	0.69
		35.83	10.42	1.14			33.3	12.43	1.1
	3	39.83	7.36	1.14		3	42.97	7.82	2.54
		38.27	7.67	0.57			35.3	11.94	0.79
		41.4	7.76	2.12			35.41	12.77	0.99
		45.47	8.6	4.03			36.38	13.22	2.28
		37.28	8.34	0.41			39.47	11.77	2.3
		37.14	8.44	0.43			42.06	7.7	1.73
	4	36.5	9.98	0.82		4	39.88	10.36	2.42
		35.96	10.43	1.1			36.86	10.75	1.13
		42.24	9.9	3.5			37.11	13.04	2.3
		37.09	9.85	1.77			37.08	13.58	2.86
		37.59	9.33	0.31			34.35	12.05	0.46
		37.18	9.71	0.78			36.02	12.03	1.18
	5	45.42	8.19	3.47		5	36.45	12.65	1.93
		40.92	8.63	2.15			37.1	12.47	1.65
		40.43	8.79	1.72			34.54	12.9	1.4
		46.26	10.99	5.33			43.68	8.49	2.82
		36.13	10.41	0.9			36.96	12.34	1.94
		38.27	9.2	1.08			34.86	13.37	1.62
	6	36.39	8.37	0.23		6	35.26	12.27	1.35
		37.79	10.27	2.88			39.28	9.64	1.98
		38.49	10.84	2.72			32.37	12.82	0.84
		34.81	10.31	0.08			36.41	11.87	2.9
		41.21	8.85	3.47			34.46	12.95	1.12
		35.95	9.35	0.25			33.74	12.52	0.84

muscle	carcass	L	a	b
LD	1	32.61	10.38	-0.77
		31.63	12.54	-0.69
		33.46	11.41	0.44
		36.89	7.49	-6.78
		35.56	6.9	-5.77
		37.72	5.5	-7.79
		32.25	10.75	0.02
	2	35.43	11.69	0.35
		35.41	11.16	0.69
		34.38	11.04	-0.14
		33.11	10.11	-0.9
		35.1	11.33	0.03
		33.67	10.45	-0.27
		32.92	11.11	-0.26
	3	38.4	10.11	1.83
		34.63	8.93	-0.35
		37.66	9.56	1.09
		36.78	9.23	0.53
		34.85	8.75	-0.11
		36.68	8.36	1.32
	4	30.53	10.53	-0.89
		33.11	10.74	-0.02
		30.65	10.52	-0.89
		34.02	12.37	0.42
		31.75	10.62	-0.17
		31.43	10.93	-0.15
	5	36.47	11.19	0.9
		37.22	10.7	1.46
		34.12	10.31	0.53
		33.09	10.96	0.59
		34.6	10.39	0.41
		33.13	8.66	-0.98
	6	31.76	10.22	-1.4
		31.15	10.27	-1.56
		33.07	9.82	0
		31.25	10.22	-1.22
		34.17	11.09	0.93
		32.99	11.51	0.82

B.3 Values of muscle weights at pre-cooking and after-cooking after 7 days' aging with calculated cooking loss

Muscle	Carcass	Weight before cooking (g)	Weight after cooking (g)	Cooking loss (%)
LD	1	182.03	139.81	23.19
	2	184.32	148.55	19.41
	3	154.45	120.95	21.69
	4	227.78	177.2	22.21
	5	176.29	135.2	23.31
	6	146.08	110.53	24.34
SM	1	75.51	54.98	27.19
	2	70.05	49.79	28.92
	3	69.56	47.78	31.31
	4	100.13	72.38	27.71
	5	91.81	65.46	28.70
	6	69.81	49.04	29.75
ST	1	30.64	24.07	21.44
	2	26.13	20.28	22.39
	3	30.36	23	24.24
	4	36.55	29.84	18.36
	5	36.89	29.59	19.79
	6	31.26	25.66	17.91
IS	1	41.38	33.25	19.65
	2	28.32	23.48	17.09
	3	51.61	42.5	17.65
	4	57.14	49.21	13.88
	5	46.62	37.45	19.67
	6	43.78	36.11	17.52
SS	1	48.96	37.81	22.77
	2	42.7	32.13	24.75
	3	43.52	32.33	25.71
	4	48.07	36.07	24.96
	5	51.4	36.87	28.27
	6	38.29	27.71	27.63

B.4 Values of the peak force (kPa) of five lamb muscles after 7 days' aging (10~12 instrumental replicates for larger muscles like LD, SM, SS and 6~8 instrumental replicates on smaller muscles like IS and ST) with calculated shear force (N)

muscle	carcass	Peak force (kPa)	Shear force (N)	Carcass	Peak force (KPa)	Shear force (N)
LD	1	62	111.3476	4	35	54.194
		34	52.0772		66	119.8148
		39	62.6612		41	66.8948
		42	69.0116		38	60.5444
		44	73.2452		33	49.9604
		26	35.1428		40	64.778
		24	30.9092		56	98.6468
		27	37.2596		64	115.5812
		57	100.7636		43	71.1284
		43	71.1284		39	62.6612
		38	60.5444		57	100.7636
		25	33.026		40	64.778
	2				76	140.9828
		41	66.8948		31	45.7268
		57	100.7636		83	155.8004
		41	66.8948		32	47.8436
		49	83.8292		61	109.2308
		42	69.0116	5	80	149.45
		52	90.1796		35	54.194
		67	121.9316		55	96.53
		43	71.1284		50	85.946
		37	58.4276		32	47.8436
		46	77.4788		34	52.0772
	3	49	83.8292	6	31	45.7268
		54	94.4132		36	56.3108
		50	85.946		53	92.2964
		41	66.8948		50	85.946
		31	45.7268		40	64.778
		39	62.6612		40	64.778
		36	56.3108		40	64.778
		50	85.946		61	109.2308
		38	60.5444		73	134.6324
		30	43.61		56	98.6468

muscle	carcass	Peak force (kPa)	Shear force (N)	Carcass	Peak force (KPa)	Shear force (N)
SM	1	27	37.2596	4	32	47.8436
		28	39.3764		67	121.9316
		35	54.194		41	66.8948
		35	54.194		31	45.7268
		39	62.6612		64	115.5812
		37	58.4276		40	64.778
		36	56.3108		71	130.3988
		67	121.9316		41	66.8948
		54	94.4132		56	98.6468
		54	94.4132		50	85.946
		34	52.0772		73	134.6324
		66	119.8148		56	98.6468
	2	64	115.5812	5	61	109.2308
		50	85.946		56	98.6468
		56	98.6468		45	75.362
		75	138.866		45	75.362
		39	62.6612		36	56.3108
		38	60.5444		47	79.5956
		61	109.2308		72	132.5156
		43	71.1284		39	62.6612
		44	73.2452		29	41.4932
		61	109.2308		59	104.9972
					47	79.5956
	3	28	39.3764	6	72	132.5156
		48	81.7124		76	140.9828
		41	66.8948		68	124.0484
		56	98.6468		76	140.9828
		57	100.7636		74	136.7492
		57	100.7636		68	124.0484
		58	102.8804		30	43.61
		68	124.0484		58	102.8804
		44	73.2452		50	85.946
		27	37.2596		69	126.1652
		43	71.1284		61	109.2308

muscle	carcass	Peak force (kPa)	Shear force (N)	Carcass	Peak force (KPa)	Shear force (N)
SS	1	26	35.1428	4	43	71.1284
		30	43.61		49	83.8292
		40	64.778		39	62.6612
		31	45.7268		46	77.4788
		32	47.8436		38	60.5444
		31	45.7268		57	100.7636
		30	43.61		56	98.6468
		28	39.3764		54	94.4132
		27	37.2596		41	66.8948
		24	30.9092		47	79.5956
					40	64.778
	2	45	75.362	5	35	54.194
		49	83.8292		53	92.2964
		54	94.4132		45	75.362
		39	62.6612		38	60.5444
		33	49.9604		45	75.362
		46	77.4788		48	81.7124
		56	98.6468		40	64.778
		36	56.3108		46	77.4788
		42	69.0116		38	60.5444
		31	45.7268		42	69.0116
					40	64.778
	3	35	54.194	6	39	62.6612
		23	28.7924		39	62.6612
		29	41.4932		49	83.8292
		25	33.026		47	79.5956
		25	33.026		40	64.778
		32	47.8436		49	83.8292
		30	43.61		28	39.3764
		30	43.61		40	64.778
		35	54.194		51	88.0628
		24	30.9092		54	94.4132
					53	92.2964